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Interactions of glyphosate and dicamba in controlling key weed species

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INTERACTIONS OF GLYPHOSATE AND DICAMBA IN CONTROLLING KEY WEED SPECIES

By

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Interest and research on herbicide-resistant cropping systems has increased dramatically since the introduction of glyphosate-resistant crops in 1996. New advances in herbicide-resistant cropping systems, such as dicamba-resistant soybean and cotton, provide opportunities to help alleviate selection pressure currently applied by glyphosate-only systems. While there is no doubt dicamba-resistant genetics will have a huge impact on production practice, there are questions that must be answered about possible interactions with dicamba and glyphosate tank mixtures. The primary objectives of this research were to evaluate the effect of glyphosate/dicamba combinations on common Mississippi weed species, as well as determine effects of these combinations on absorption and translocation of dicamba.

Four monocots: johnsongrass, barnyardgrass, large crabgrass, and broadleaf signalgrass; and four dicots: sicklepod, hemp sesbania, prickly sida, and pitted morningglory, were chosen to represent troublesome weed species. Plants were sprayed at the 4±1 leaf stage with glyphosate, dicamba, and

combinations of the two herbicides. Rates were chosen with the goal of achieving 40 to 70% control in order to determine synergistic/antagonistic responses. Antagonism was observed in each species tested. Increasing rates of both herbicides alleviated the antagonism in most weeds. A synergistic response was observed in all graminaceous species and pitted morningglory when herbicide rates increased.

Barnyardgrass and sicklepod were selected to quantify absorption and translocation of ^{14}C-dicamba in order to account for interactions observed from tank-mix combinations. Rates for dicamba and glyphosate were selected based on results from the interaction study. Dicamba, glyphosate, and tank-mix combinations were applied to sicklepod and barnyardgrass before treatment with ^{14}C-dicamba. Plants were harvested 4, 12, 24, and 72 h after treatment. The addition of glyphosate to dicamba resulted in reduced translocation of ^{14}C-dicamba in both species. While the data did indicate a translocation interaction, glyphosate and dicamba combinations effectively overcame antagonism effects when higher rates were applied on sicklepod and barnyardgrass.

DEDICATION

This manuscript is a culmination of many years and the hard work of many people. I feel it would be a dishonor to only dedicate this to one person when so many have molded the person I am today. I would like to thank my family members: Bill Huff, Vicki Henderson, Stephanie Etheridge, and Chris Huff. Without your support, patience, kindness, and encouragement, I would not have made it to this day. I could not ask for a better group of people to call my family. Thank you for standing beside me these 28 long years. I hope that I can always be there for each of you, the way you have been there for me.

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CHAPTER I

INTRODUCTION

The ability of a herbicide to be an effective resource for weed management depends on the susceptibility of target weed species to specific modes of action and herbicidal properties (Devine et al. 1993). Herbicides are applied at various times throughout a growing season to optimize efficacy on target weed species and limit crop injury (Radosevich et. al 1997). Many herbicides are currently used in agronomic cropping systems, but no herbicide has been utilized as frequently as glyphosate over the past 15 years.

Glyphosate is a broad-spectrum, foliar-applied herbicide that rapidly translocates from treated foliage to metabolically active regions of roots, rhizomes, and apical meristem (Franz 1985; Kishore et al. 1992). The ability of glyphosate to be an effective herbicide depends on the type of surfactant (Kirkwood 1993; Haztios and Penner 1985), rate of application (Ambach and Ashford 1982), and water quality (Nalewaja and Atysiak 1993). Glyphosate efficacy can also be affected by interaction with other herbicides (Hydrick and Shaw 1994; Jordan et al. 1997), weed species (Flint and Barrett 1989a; Flint and Barrett 1989b) and size (Parker et al. 2006). The interaction of glyphosate with other herbicides can cause antagonistic responses with respect to weed control (Flint and Barrett 1989a; Selleck and Baird 1981).

The introduction of glyphosate-resistant (GR) crops in 1996 fundamentally changed agricultural systems (Owen 2000). Since the introduction of these GR cropping systems, glyphosate usage has increased exponentially. The increase in glyphosate usage can be attributed to development and rapid adoption of not only GR soybean [*Glycine max* (L.) Merr.], but GR cotton (*Gossypium hirsutum* L.) and GR corn (*Zea mays* L.) as well. GR cropping systems encompass 68, 65, and 91% of total U.S. crops hectarage in corn, cotton, and soybean, respectively (Anonymous 2009).

In recent years, glyphosate-resistant weeds and their management have come to the forefront of weed research. Currently there are 17 species listed worldwide with glyphosate resistance: buckhorn plantain (*Plantago lanceolata L.*), common ragweed (*Ambrosia artemisiifolia* L.), common waterhemp (*Amaranthus rudis* Sauer), giant ragweed (*Ambrosia trifida* L.), goosegrass [*Eleusine indica* (L.) Gaertn.], hairy fleabane [*Conyza bonariensis* (L.) Cronq.], horseweed [*Conyza canadensis* (L.) Cronq.], Italian ryegrass [*Lolium perenne* L. *ssp. multiflorum* (Lam.) Husnot], johnsongrass [*Sorghum halepense* (L.) Pers.], junglerice [*Eichinochloa colona* (L.) Link], kochia [*Kochia scoparia (L.) Schrad.*], liverseedgrass (*Urochloa panicoides* Beauv.), Palmer amaranth (*Amaranthus palmeri* S. Wats.), ragweed parthenium (*Parthenium hysterophorus L.*), rigid ryegrass (*Lolium rigidum* Gaudin), sourgrass [*Digitaria insularis (L.)* Mez ex Ekman], and wild poinsettia (*Euphorbia heterophylla L.*) (Heap, 2010). The number of new GR weed species has averaged close to one per year since the introduction of GR crops. The resistance issues are not limited to one country or geographic region. Currently 11 countries have reported glyphosate resistance in various weed species.

Advances have been made to create cropping systems that are tolerant to various herbicide modes-of-action other than glyphosate. Dicamba/glyphosate-resistant soybean and cotton cultivars are two examples of new genetically modified organisms that provide alternatives to glyphosate systems (Behrens et al. 2007). Incorporation of dicamba tolerance in plants has potential to introduce new modes-of-action into these cropping systems, helping provide new solutions for weed control (Subramanian et al. 1997). Dicamba is an auxin-mimicking herbicide used for postemergence control of dicot weeds in corn and wheat (*Triticum aestivum* L.) (Tomlin 1994). Dicamba is a synthetic auxin that mimics the natural plant hormone indole-3-acetic acid, causing an epinastic response in target weed species, eventually leading to chlorosis and necrosis (WSSA 2007).

Dicamba is an effective herbicide, but several questions are raised with regards to tank-mixing dicamba with glyphosate. Glyphosate has produced antagonistic and synergistic responses when tank-mixed with different herbicides (Hydrick and Shaw 1994; Selleck and Baird 1981). Flint and Barrett (1989b) reported a synergistic response on field bindweed (*Convolvulus arvensis* L.) to applications of glyphosate plus dicamba. However, Flint and Barrett (1989a) reported an antagonistic response on johnsongrass when glyphosate and dicamba were applied together. Dicamba and glyphosate tank-mix combinations reduce control on several graminaceae weeds (O'Sullivan and O'Donovan 1980).

Flint and Barrett (1989a and 1989b) reported deviations in glyphosate efficacy related to absorption and translocation patterns when tank-mixed with dicamba.

The objectives of the research reported in the following chapters were to evaluate the effect of glyphosate/dicamba combinations on common Mississippi weed species, as well as to determine effects of this combination on absorption and translocation of dicamba in the plant.

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CHAPTER II

WEED CONTROL FROM TANK-MIX COMBINATIONS OF DICAMBA AND GLYPHOSATE

Abstract

The development of dicamba-resistant crops opens a variety of opportunities to more effectively manage weeds in these crops. However, questions have arisen regarding possible interactions between glyphosate and dicamba herbicide combinations. The objective of this research was to determine the potential for synergistic or antagonistic effects of various rates of glyphosate plus dicamba tank-mix combinations on a variety of common weeds. Three monocots: broadleaf signalgrass, johnsongrass, large crabgrass; and three dicots: hemp sesbania, pitted morningglory, and prickly sida, were treated at the 4±1 leaf stage with various rates of dicamba, glyphosate, and tank-mix combinations of the two herbicides. Antagonistic effects were observed in all species when low rates of dicamba were applied with low rates of glyphosate. Tank-mix combinations of low rates of dicamba and glyphosate decreased control of broadleaf signalgrass, johnsongrass, and large crabgrass compared with glyphosate alone. Antagonism was no longer observed in broadleaf signalgrass, johnsongrass, or large crabgrass when 0.42 or 0.56 kg ae ha^{-1} dicamba was tank-mixed with any rate of glyphosate. Tank-mix combinations of low rates of dicamba combined with any rate of glyphosate provided an antagonistic response on hemp sesbania and pitted morningglory. Hemp sesbania control was reduced with tank-mix combinations of 0.14 kg ha^{-1} dicamba and 0.56 kg ha^{-1} glyphosate, 0.28 kg ha^{-1} dicamba plus 0.56 kg ha^{-1} glyphosate, and 0.42 kg ha^{-1} dicamba plus 0.28 kg ha^{-1} glyphosate compared with dicamba alone. Similarly, pitted morningglory control was decreased when 0.28 kg ha^{-1} dicamba was applied in tank-mix combination with 0.28 kg ha^{-1} glyphosate compared with glyphosate alone. Antagonistic effects were no longer observed in hemp sesbania or pitted morningglory when 0.56 kg ha^{-1} dicamba was combined with any rate of glyphosate. Antagonism occurred with prickly sida when low rates of glyphosate were combined with any rate of dicamba. Antagonistic effects were no longer observed in prickly sida when glyphosate rates were increased to 0.84 or 1.12 kg ha^{-1}. These greenhouse studies indicated a strong potential for antagonistic interactions when dicamba and glyphosate are tank-mixed; thus, care should be taken to use rates of these herbicides that can overcome this antagonism.

Nomenclature: dicamba, glyphosate, broadleaf signalgrass, *Urochloa platyphylla* (Nash) R.D. Webster; hemp sesbania, *Sesbania herbacea* (P. Mill.) McVaugh.; johnsongrass, *Sorghum halepense* (L.) Pers.; large crabgrass, *Digitaria sanguinalis* (L.) Scop.; pitted morningglory, *Ipomoea lacunosa* L.; prickly sida, *Sida spinosa* L.

Introduction

Glyphosate is considered by many as the most important herbicide ever developed (Powles 2003). Glyphosate's broad-spectrum activity on monocot and dicot weeds and favorable environment characteristics have led to improved yields, increases in conservation tillage systems, and higher quality agricultural products (Gianessi and Sankula 2004). The glyphosate mode of action is unique to the shikimic acid pathway that plants inherently possess (Grossbard and Atkinson 1985). Glyphosate inhibits 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), which produces EPSP from shikimate-3-phosphate and phosphoenolpyruvate in the shikimic acid pathway (WSSA 2007). The inhibition of EPSPS leads to depletion of the aromatic amino acids tryptophan, tyrosine, and phenylalanine. These aromatic amino acids are precursors that lead to the creation of secondary metabolites within the plant (WSSA 2007). This unique mode of action, coupled with limited selection pressure during the first 20 years of glyphosate usage, disfavored the development of glyphosate resistance (Mueller et al. 2005; Powles and Preston 2006). Powles (2008) also cited glyphosate's lack of residual activity in the environment and incorporation of diverse weed control practices prior to the adoption of glyphosate-resistant (GR) crops as factors contributing to the absence of evolved glyphosate resistance during the earlier of herbicide use.

The introduction of GR crops in 1996 fundamentally changed agronomic systems (Owen 2000). With the development of GR technology in cotton (*Gossypium hirsutum* L.), corn (*Zea mays* L.), and soybean [G*lycine max* (L.)

Merr], glyphosate usage has increased dramatically in the U.S. and worldwide (Owen 2000; Powles and Preston 2006). Thus, it is now not only the predominant herbicide used in burndown/non-crop applications; it is also the most common product used in-season in the aforementioned crops (Duke and Powles 2008). The massive increase of glyphosate usage associated with GR cropping systems has placed intense selection pressure on target weed species. While Pratley et al. (1999) and Powles et al. (1998) reported the first instances of evolved glyphosate resistance in rigid ryegrass (*Lolium rigidum* Gaudin), VanGessel (2001) reported the first instance of evolved glyphosate resistance in a GR crop setting with horseweed [*Conyza canadensis* (L.) Cronq.].

In recent years, glyphosate resistance management has come to the forefront of weed research. Currently there are 17 species listed worldwide with glyphosate resistance: buckhorn plantain (*Plantago lanceolata L.*), common ragweed (*Ambrosia artemisiifolia* L.), common waterhemp (*Amaranthus rudis* Sauer), giant ragweed (*Ambrosia trifida* L.), goosegrass [*Eleusine indica* (L.) Gaertn.], hairy fleabane [*Conyza bonariensis* (L.) Cronq.], horseweed [*Conyza canadensis* (L.) Cronq.], Italian ryegrass [*Lolium perenne* L. *ssp. multiflorum* (Lam.) Husnot], johnsongrass [*Sorghum halepense* (L.) Pers.], junglerice [*Eichinochloa colona* (L.) Link], kochia [*Kochia scoparia (L.) Schrad.*], liverseedgrass (*Urochloa panicoides* Beauv.), Palmer amaranth (*Amaranthus palmeri* S. Wats.), ragweed parthenium (*Parthenium hysterophorus L.*), rigid ryegrass (*Lolium rigidum* Gaudin), sourgrass [*Digitaria insularis (L.)* Mez ex Ekman], and wild poinsettia (*Euphorbia heterophylla L.*) (Heap 2010). The development of glyphosate resistance has renewed interest in diverse weed management strategies, such as rotation of herbicide modes of action, crop rotation, and tillage systems (Jasieniuk et al. 1996; Koger et al. 2005; Mueller et al. 2005; Powles 2003; Shaw et al. 2009).

Advances have been made to create cropping systems that impart crop selectivity to various herbicide modes of action other than glyphosate. The development of dicamba/glyphosate-resistant soybean and cotton can provide an alternative mode of action to GR cropping systems (Behrens et al. 2007; Subramanian et al. 1997). Dicamba is a synthetic auxin that mimics the natural plant hormone indole-3-acetic acid, causing an epinastic response in target weed species, eventually leading to chlorosis and necrosis (WSSA 2007). The use of dicamba in cropping systems has typically been for broadleaf weed control before planting (Everitt and Keeling 2007) or in graminaceous crops (Tomlin 1994). The advent of the new dicamba biotechnology creates the opportunity to increase herbicide diversity by allowing tank-mix combinations (Behrens et al. 2007). However, questions remain regarding tank-mixing dicamba with glyphosate. Flint and Barrett (1989a) observed a synergistic response on field bindweed (*Convolvulus arvensis* L.) to applications of glyphosate and dicamba combinations, but also reported (1989b) an antagonistic response on johnsongrass when the same combination was applied. O'Sullivan and O'Donovan (1980) also found reduced control on several graminaceous weeds when combinations of glyphosate and dicamba were applied. Currently, dicamba is registered as a tank-mix partner with glyphosate (BASF 2010). However, further investigations are needed to measure potential interactions of tank-mix combinations of glyphosate and dicamba.

The objective of this research was to evaluate the effect of glyphosate/dicamba combinations on common weed species.

Materials and Methods

Seeds of broadleaf signalgrass, hemp sesbania, johnsongrass, large crabgrass, pitted morningglory, and prickly sida were placed in 9-cm^2 pots containing Metro-Mix 300 (horticulture grade vermiculite, bark, Canadian sphagnum peat moss, horticulture grade perlite, processed bark ash, starter nutrient charge, dolomitic limestone, and wetting agent). Species chosen were listed as some of the most troublesome weeds in Mississippi cropping systems (Anonymous 2010). Plants were grown in a greenhouse with 35/30 C day/night temperatures and were surface-irrigated daily to provide adequate moisture. Supplemental lighting was provided by sodium vapor lamps to provide a 16 h photoperiod. Within one week of emergence, plants were thinned to one plant per pot. Plants were treated at the 4 ± 1 leaf stage. Plants chosen for treatment were treated at a larger-than-optimum size, with herbicide rates determined from previous dicamba plus glyphosate tank-mix studies (Flint and Barrett 1989a; Flint and Barrett 1989b) to amplify differences between herbicide treatments.

The experimental design was a randomized complete block with a factorial arrangement of treatments consisting of 0.14, 0.28, 0.42, and 0.56 kg ha^{-1} of the diglycolamine salt of dicamba, and 0.28, 0.56, 0.84, and 1.12 kg ha^{-1} of the isopropylamine salt of glyphosate. Factors were herbicide and herbicide rate. An untreated check was also included for comparison. Experiments were conducted twice with each treatment replicated four times. All herbicide rates were applied individually and in combination in a compressed-air spray chamber equipped with an XR110015E flat fan nozzle at an application volume of 169 L ha^{-1}. Dicamba treatments applied individually included 391A, a proprietary surfactant, at 0.5% (v/v) to equalize surfactant effects. Plants were harvested and fresh weight taken 21 days after treatment.

Interactions between treatments were calculated utilizing methods described by Colby (1967) (Hydrick and Shaw 1994; Koger et al. 2005; Koger et al. 2007). This method compares observed percent reduction values of herbicide combinations to expected percent reduction values calculated from percent reduction of the herbicides applied alone.

Expected percent reduction values are calculated as followed:

$$E= X-Y (XY/100) \tag{2-1}$$

where E is the expected value, X is equal to the percent inhibition of growth by herbicide A at p kg ha^{-1}, and Y is equal to the percent inhibition of growth by herbicide B at p kg ha^{-1}. Expected and observed values were compared by Fisher's protected LSD at the 0.05 level of significance. Herbicide interactions were considered synergistic if the observed response was greater than the expected response, antagonistic if the observed response was less than the expected response, and additive if there was no difference between the expected and observed values.

Results and Discussion

Broadleaf signalgrass fresh weight reduction ranged from 51 to 100% with glyphosate and 13 to 32% with dicamba (Table 2.1). Antagonism occurred when 0.14 kg ha^{-1} dicamba was applied with any rate of glyphosate and when 0.28 kg ha^{-1} dicamba was applied with 0.84 kg ha^{-1} glyphosate. However, increasing the rate of dicamba to 0.42 or 0.56 kg ha^{-1} improved control of broadleaf signalgrass, resulting in a synergistic effect when applied with glyphosate at 0.28 or 0.56 kg ha^{-1}. Dicamba at 0.42 and 0.56 kg ha^{-1} tank-mixed with either 0.84 or 1.12 kg ha^{-1} glyphosate negated any antagonistic observations from lower rates of the tank-mix combination, resulting in an additive effect with control ranging from 96 to 99%. Tank-mix combinations of 0.14 kg ha^{-1} dicamba and any rate of glyphosate, 0.28 kg ha^{-1} dicamba, and either 0.84 kg ha^{-1} glyphosate, and 0.42 kg ha^{-1} dicamba and 0.84 kg ha^{-1} glyphosate reduced control of broadleaf signalgrass compared with glyphosate alone.

Hemp sesbania fresh weight reduction ranged from 79 to 91% with dicamba and 51 to 84% with glyphosate (Table 2.2). Antagonism occurred when 0.14, 0.28. or 0.42 kg ha^{-1} dicamba was combined with any rate of glyphosate, excluding the additive effect observed from the combination of 0.42 kg ha^{-1} dicamba and 1.12 kg ha^{-1} glyphosate. Koger et al. (2007) reported antagonism with tank-mix combinations of glyphosate and MSMA on hemp sesbania. Increasing the rate of dicamba to 0.56 kg ha^{-1} across all rates of glyphosate eliminated observed antagonism. Tank-mix combinations of 0.14 kg ha^{-1} dicamba and 0.56 kg ha^{-1} glyphosate, 0.28 kg ha^{-1} dicamba and 0.28 kg ha^{-1} glyphosate, and 0.42 kg ha^{-1} dicamba and 0.28 kg ha^{-1} glyphosate reduced control of hemp sesbania compared to dicamba alone.

Johnsongrass fresh weight reduction ranged from 44 to 89% with glyphosate and 9 to 12% with dicamba (Table 2.3), similar to findings of Flint and Barrett (1989b). Antagonism was observed when 0.14 kg ha^{-1} dicamba was applied with any rate of glyphosate. This antagonistic interaction resulted in fresh weight reductions of 24 to 76 percentage points less than glyphosate alone. An additive effect was observed with 0.28 kg ha^{-1} dicamba tank-mixed with 0.28 kg ha^{-1} glyphosate; however, antagonism was reestablished with glyphosate rates of 0.56, 0.84, and 1.12 kg ha^{-1}. Reductions resulting from the aforementioned antagonistic tank-mix combinations were 8 to 23 percentage points less than glyphosate alone at the same rate. Conversely, when dicamba rates increased to 0.42 and 0.56 kg ha^{-1}, antagonistic effects were no longer observed with any combinations. These results slightly differ from those of Flint and Barrett (1989b), who noted antagonism with tank-mix combinations of 0.42 and 0.56 kg ha^{-1} dicamba and 0.28 and 0.56 kg ha^{-1} glyphosate. However, both studies report the absence of antagonism when combinations of 0.42 and 0.56 kg ha^{-1} dicamba were combined with the higher rates, 0.84 and 1.12 kg ha^{-1}, of glyphosate. Thus, the potential for antagonism is clearly demonstrated in both studies.

Large crabgrass fresh weight reduction reached 99% when glyphosate was applied at a rate of 1.12 kg ha^{-1} (Table 2.4). Dicamba applied alone produced no more than 17% fresh weight reduction. When 0.14 kg ha^{-1} dicamba was applied with all rates of glyphosate, antagonism occurred. Fresh weight reductions were 4 to 50 percentage points lower compared with glyphosate applied alone. The tank-mix combination of 0.42 kg ha^{-1} dicamba and 0.84 kg ha^{-1} glyphosate also resulted in a decrease in fresh weight reduction of 10 percentage points compared to glyphosate applied alone. Tank-mixing 0.28 kg ha^{-1} dicamba with 0.84 or 1.12 kg ha^{-1} glyphosate also resulted in antagonism and less fresh weight reduction, 50 and 49 percentage points, respectively. However, increasing the rate of dicamba to 0.42 or 0.56 kg ha^{-1} resulted in a synergistic response when tank-mixed with 0.28 and 0.56 kg ha^{-1} of glyphosate. The tank-mix combination of 0.42 kg ha^{-1} dicamba and 0.84 kg ha^{-1} glyphosate produced an antagonistic effect, and reduced fresh weight than glyphosate applied alone. Increasing the rate of dicamba to 0.56 kg ha^{-1} eliminated antagonism for all glyphosate tank-mix combinations and resulted in an additive effect ranging, from 99 to 100% fresh weight reduction.

Pitted morningglory fresh weight reduction ranged from 1 to 91% with glyphosate and 3 to 74% with dicamba when herbicides were applied alone (Table 2.5). Variable control of pitted morningglory with glyphosate has been reported previously (Shaw and Arnold 2002; Koger et al. 2007). Combinations of

0.14 or 0.28 kg ha^{-1} dicamba with glyphosate indicated antagonism, except the combination of 0.14 kg ha^{-1} dicamba and 0.28 kg ha^{-1} glyphosate (which exhibited very minimal control). Antagonism was also observed when 0.42 kg ha^{-1} dicamba was applied with 1.12 kg ha^{-1} glyphosate. Antagonistic tank-mix combinations exhibited a 9 to 39 percentage point decrease in fresh weight reduction compared with glyphosate alone. Tank-mix combinations of 0.28 kg ha^{-1} dicamba and 0.28 kg ha^{-1} glyphosate decreased control of pitted morninggglory when compared to dicamba alone. Synergistic effects were observed when 0.42 kg ha^{-1} dicamba was applied with 0.28 or 0.56 kg ha^{-1} glyphosate. Dicamba at 0.56 kg ha^{-1} tank-mixed with 0.28 or 0.56 kg ha^{-1} glyphosate resulted in a synergistic effect. However, tank-mix combinations of 0.56 kg ha^{-1} dicamba with 0.84 or 1.12 kg ha^{-1} exhibited an additive effect.

Prickly sida control ranged from 54 to 99% with glyphosate and 57 to 73% with dicamba when the herbicides were applied alone (Table 2.6). Antagonistic effects were observed when 0.28 or 0.56 kg ha^{-1} glyphosate were tank-mixed with any rate of dicamba. When glyphosate rates increased to 0.84 and 1.12 kg ha^{-1}, antagonistic effects were no longer observed with fresh weight reduction ranging from 91 to 100%; this included a synergistic response to the tank-mix of 0.28 kg ha^{-1}dicamba and 1.12 kg ha^{-1} glyphosate. Increasing rates of non-selective herbicides can overcome antagonism of tank-mix combinations when selective herbicide rates remain the same (Hydrick and Shaw 1994; O'Donovan and O'Sullivan 1982).

The results of this study indicate that tank-mix combinations of glyphosate and dicamba can result in either synergism or antagonism, depending on species and herbicide rate. Peterson et al. (1974) reported decreased translocation of auxin-like herbicides due to leakage of herbicide into the vascular parenchyma resulting in physical constriction with the plant pathways. Flint and Barrett (1989b) reported reductions of glyphosate absorption and translocation with johnsongrass when applied with dicamba, leading to the assumption that herbicide components other than glyphosate were responsible for the observed reductions. Flint and Barrett (1989a) also found that dicamba and glyphosate combinations had additive or synergistic effects on field bindweed roots systems, resulting from decreased translocation of glyphosate to the apical meristem and increased concentrations observed in the roots. There are also a number of other factors that must be considered when attempting to develop weed management strategies with various herbicides or herbicide combinations. Glyphosate efficacy can be affected by interaction with other herbicides (Jordan et al. 1997; Hydrick and Shaw 1994), weed species (Flint and Barrett 1989a; Flint and Barrett 1989b) and size (Parker et al., 2006). If herbicide tank-mix partners do not provide similar efficacy/persistence, offer different propensities for selecting for resistance in target species, and result in synergistic effects, then applications of glyphosate-alternative herbicide mixtures will not be an effective means for reducing selection pressure in a diverse weed population (Beckie 2006; Boerboom 2007). Although increasing application rates can effectively eliminate some antagonism, in certain situations higher rates may not be feasible

(Hydrick and Shaw 1994). This research has shown that with the incorporation of dicamba tolerance in plants as an option for future cropping system, questions must be answered about implications of dicamba and glyphosate tank mixtures. The intention of this paper was to provide an assessment of potential herbicide interactions and identify possible problematic areas associated with herbicide antagonism with these two compounds. Further research is needed to determine the basis for these herbicide interactions on a weed-by-weed basis.

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Table 2.1. Percent reduction in fresh weight of broadleaf signalgrass 21 days after postemergence treatments with tank-mix combinations of dicamba and glyphosate.

Glyphosate	Dicamba rate (kg ae/ha) [a]				
rate	0.0	0.14	0.28	0.42	0.56
(kg ae/ ha)	%				
0.0		13	11	26	32
0.28	51	41 (57)- [b, c]	57 (56)	95 (64)+	96 (67)+
0.56	56	42 (71)-	77 (70)	97 (75)+	99 (77)+
0.84	100	60 (100)-	74 (100)-	96 (100)	99 (100)
1.12	100	62 (100)-	94 (100)	98 (100)	98 (100)
LSD [d]	8				

[a] Dicamba treatment applied alone contained 0.05% 391A.

[b] A negative sign denotes an antagonistic response; a positive sign denotes a synergistic response.

[c] Values in parentheses are the calculated (expected) level of percent fresh weight reduction for the herbicide combinations.

[d] LSD: Least significant difference separated by Fishers protected LSD at the 0.05 level of significance.

Table 2.2. Percent reduction in fresh weight of hemp sesbania 21 days after postemergence treatments with tank-mix combinations of dicamba and glyphosate.

Glyphosate rate	Dicamba rate (kg ae/ha)[a]				
	0.0	0.14	0.28	0.42	0.56
(kg ae/ ha)	%				
0.0		79	86	91	87
0.28	51	77 (90)-[b, c]	80 (95)-	82 (96)-	95 (93)
0.56	56	73 (91)-	83 (95)-	87 (96)-	97 (95)
0.84	68	76 (93)-	86 (96)-	87 (97)-	98 (96)
1.12	84	79 (97)-	82 (98)-	94 (98)	100 (98)
LSD[d]	5				

[a] Dicamba treatment applied alone contained 0.05% 391A.

[b] A negative sign denotes an antagonistic response; a positive sign denotes a synergistic response.

[c] Values in parentheses are the calculated (expected) level of percent fresh weight reduction for the herbicide combinations.

[d] LSD: Least significant difference separated by Fishers protected LSD at the 0.05 level of significance.

Table 2.3. Percent reduction in fresh weight of johnsongrass 21 days after postemergence treatments with tank-mix combinations of dicamba and glyphosate.

Glyphosate rate	Dicamba rate (kg ae/ha) [a]				
	0.0	0.14	0.28	0.42	0.56
(kg ae/ ha)	%				
0.0		9	12	10	11
0.28	44	20 (49)- [b, c]	46 (51)	80 (50)+	86 (50)+
0.56	72	28 (75)-	67 (75)-	82 (75)+	86 (75)+
0.84	78	15 (81)-	68 (81)-	84 (81)	88 (81)+
1.12	89	13 (88)-	66 (89)-	84 (89)	88 (90)
LSD [d]	5				

[a] Dicamba treatment applied alone contained 0.05% 391A.

[b] A negative sign denotes an antagonistic response; a positive sign denotes a synergistic response.

[c] Values in parentheses are the calculated (expected) level of percent fresh weight reduction for the herbicide combinations.

[d] LSD: Least significant difference separated by Fishers protected LSD at the 0.05 level of significance.

Table 2.4. Percent reduction in fresh weight of large crabgrass 21 days after postemergence treatments with tank-mix combinations of dicamba and glyphosate.

Glyphosate	Dicamba rate (kg ae/ha) [a]				
rate	0.0	0.14	0.28	0.42	0.56
(kg ae/ ha)	%				
0.0		16	16	17	10
0.28	46	42 (54)- [b, c]	50 (55)	91 (51)+	99 (51)+
0.56	48	38 (56)-	54 (56)	94 (53)+	99 (53)+
0.84	95	45 (95)-	45 (95)-	85 (95)-	100 (95)
1.12	99	52 (100)-	51 (100)-	95 (100)	99 (100)
LSD [d]	8				

[a] Dicamba treatment applied alone contained 0.05% 391A.

[b] A negative sign denotes an antagonistic response; a positive sign denotes a synergistic response.

[c] Values in parentheses are the calculated (expected) level of percent fresh weight reduction for the herbicide combinations.

[d] LSD: Least significant difference separated by Fishers protected LSD at the 0.05 level of significance.

Table 2.5. Percent reduction in fresh weight of pitted morningglory 21 days after postemergence treatments with tank-mix combinations of dicamba and glyphosate.

Glyphosate	Dicamba rate (kg ae/ha) [a]				
rate	0.0	0.14	0.28	0.42	0.56
(kg ae/ ha)			%		
0.0		3	28	40	74
0.28	1	2 (3) [b]	14 (28)- [c]	72 (40)+	85 (75)+
0.56	16	7 (18)-	30 (41)-	75 (48)+	82 (78)+
0.84	79	43 (79)-	58 (85)-	81 (87)	88 (94)
1.12	91	52 (92)-	72 (94)-	76 (95)-	91 (98)
LSD [d]			10		

[a] Dicamba treatment applied alone contained 0.05% 391A.

[b] Values in parentheses are the calculated (expected) level of percent fresh weight reduction for the herbicide combinations.

[c] A negative sign denotes an antagonistic response; a positive sign denotes a synergistic response.

[d] LSD: Least significant difference separated by Fishers protected LSD at the 0.05 level of significance.

Table 2.6. Percent reduction in fresh weight of prickly sida 21 days after postemergence treatments with tank-mix combinations of dicamba and glyphosate.

Glyphosate	Dicamba rate (kg ae/ha) [a]				
rate	0.0	0.14	0.28	0.42	0.56
(kg ae/ ha)	---------- % ----------				
0.0		57	57	60	73
0.28	54	67 (80)- [b, c]	57 (81)-	55 (82)-	73 (88)-
0.56	60	61 (92)-	78 (92)-	67 (92)-	80 (95)-
0.84	96	93 (98)	91 (98)	100 (99)	100 (99)
1.12	99	95 (100)	88 (100)+	100 (100)	100 (100)
LSD [d]	---------- 8 ----------				

[a] Dicamba treatment applied alone contained 0.05% 391A.

[b] A negative sign denotes an antagonistic response; a positive sign denotes a synergistic response.

[c] Values in parentheses are the calculated (expected) level of percent fresh weight reduction for the herbicide combinations.

[d] LSD: Least significant difference separated by Fishers protected LSD at the 0.05 level of significance.

CHAPTER III

EFFECTS OF GLYPHOSATE AND DICAMBA TANK-MIX COMBINATIONS ON BARNYARDGRASS (*Echinochloa crus-galli*)

Abstract

The development of dicamba-resistant crops opens up a variety of opportunities to more effectively manage weeds in these crops, particularly in glyphosate-resistant crops. However, questions have also arisen regarding possible interactions between glyphosate and dicamba herbicide combinations. The objectives of this research were to determine the effects of dicamba/glyphosate tank-mix combinations on barnyardgrass, and identify the physiological basis for any observed response. Barnyardgrass was treated at the 4±1 leaf stage with various rates of dicamba, glyphosate, and combinations of the two herbicides. Antagonism occurred when 0.14 and 0.28 kg ae ha^{-1} dicamba were combined with either 0.84 or 1.12 kg ae ha^{-1} glyphosate, and when 0.42 kg ha^{-1} dicamba was tank-mixed with 1.12 kg ha^{-1} glyphosate. The combinations of the aforementioned herbicides also reduced control of barnyardgrass compared with glyphosate alone. Antagonism was no longer observed with combinations of 0.28 and 0.42 kg ha^{-1} dicamba with either 0.28 or 0.56 kg ha^{-1} glyphosate, as well as 0.56 kg ha^{-1} dicamba tank-mixed with 0.28, 0.56, or 0.84 kg ha^{-1} glyphosate. Rates selected from the interaction study were

then sprayed on barnyardgrass plants prior to treatment with ^{14}C-dicamba. The addition of glyphosate to dicamba reduced ^{14}C-dicamba in plant material above the treated leaf collar and below the treated leaf collar to the soil line. ^{14}C-dicamba recovery increased incrementally over time with regards to treated leaf concentration, untreated plant material concentration, root concentration, and absorption. These data indicate that increasing rate of dicamba with glyphosate effectively controlled barnyardgrass, and that translocation of dicamba was altered when glyphosate was added in a tank-mix combination. Metabolism, blockage of plant transport systems, and herbicide interference could account for the hindrance of translocation with the tank-mix of dicamba and glyphosate. Nomenclature: dicamba, glyphosate, Barnyardgrass, [*Echinochloa crus-galli* (L.) Beauv.]

Introduction

Barnyardgrass [*Echinochloa crus-galli* (L.) Beauv.] is one of the most troublesome weeds in agronomic settings worldwide (Holm et al. 1991). Interference from barnyardgrass reduces yield in a variety of crops including: corn [*Zea mays* (L.) Merr.] (Bosnic and Swanton 1997), cotton (*Gossypium hirsutum* L.) (Keeley and Thullen 1991), rice (*Oryza sativa* L.) (Smith 1968; Smith 1988; and Smith and Khodayari 1985), and soybean [*Glycine max (*L.*)* Merr.] (Vail and Oliver 1993). Glyphosate has been used as an effective tool for controlling barnyardgrass in glyphosate-resistant (GR) crops (Jordan et al. 1996; Webster et al. 1999). Since the introduction of these GR cropping systems, glyphosate usage has increased exponentially (Powles 2003). The increase in glyphosate usage can be attributed to development and rapid incorporation of not only GR soybean, but GR cotton and GR corn as well. The incorporation of the herbicide-resistant cropping systems encompasses 68, 65, and 91% of total U.S. crops hectarage in corn, cotton, and soybean, respectively (Anonymous 2009).

Recently, advances have been made to create cropping systems that are tolerant to various herbicide modes of action other than glyphosate. Dicamba-resistant soybean and cotton cultivars are two examples of new genetically modified organisms that provide alternatives to glyphosate systems (Behrens et al. 2007). Incorporation of dicamba tolerance in plants has potential to introduce new modes of action into these cropping systems, helping provide new solutions for weed control (Subramanian et al. 1997). This new technology will allow for tank mixtures of dicamba and glyphosate for control of broadleaf and graminaceous species. Although adding dicamba to glyphosate can enhance control of various broadleaf species (Flint and Barrett 1989a), the combination of these two herbicides can have an antagonistic effect on the control of graminaceous species (Flint and Barrett 1989b). O'Sullivan and O'Donovan (1980) reported reductions in glyphosate toxicity to barley (*Hordeum vulgare* L.), wheat (*Triticum aestivum* L.), and wild oats (*Avena fatua* L.) when dicamba was added to treatment solutions. Flint and Barrett (1989b) reported reductions of glyphosate absorption and translocation when dicamba was added in spray solution, but determined the basis for the observed reductions to be with other herbicide components in the tank mixture rather than the glyphosate itself. The possibility of rapid adoption of dicamba cropping systems raises questions about the interactions between the two herbicides. To more fully understand the ramifications of glyphosate plus dicamba tank-mix combinations, studies are needed to determine efficacy on troublesome weed species such as barnyardgrass.

The objectives of this research were to determine the effects of dicamba/glyphosate tank-mix combinations on barnyardgrass, and identify the physiological basis for any observed response with dicamba.

Materials and Methods

Interaction Study

Barnyardgrass seed was planted in 9-cm^2 pots containing Metro-Mix 300 (horticulture grade vermiculite, bark, Canadian sphagnum peat moss, horticulture grade perlite, processed bark ash, starter nutrient charge, dolomitic limestone, and wetting agent) and grown at 35/30 C day/night temperature with daily surface irrigation for adequate moisture. Natural light was supplemented with light from sodium vapor lamp to provide a 16-hr photoperiod. Plants were thinned to one plant per pot within one week of emergence. Plants were selected for treatment upon development of the fourth true leaf (15 to 20 cm in height). Plants were treated at larger than optimum size due to increased sensitivity under greenhouse conditions. Herbicide rates were chosen based on labeled rates for barnyardgrass and prior antagonism studies conducted utilizing glyphosate and dicamba (Flint and Barrett 1989a; Flint and Barrett 1989b). Experiments were conducted twice, with treatments replicated four times in a randomized complete block design with a two-factor factorial arrangement of treatments. The first factor included 0.14, 0.28, 0.42, and 0.56 kg ha^{-1} dicamba. The second factor included 0.28, 0.56, 0.84, and 1.12 kg ha^{-1} glyphosate. All herbicide rates were applied individually and in combination in a compressed-air spray chamber equipped with an XR110015E flat fan nozzle at an application volume of 169 L ha^{-1}. Dicamba treatments applied individually included 391A, a proprietary surfactant from Helm Agro U.S., at 0.5% (v/v) to equalize surfactant effects. Plants were harvested and fresh weight taken 21 days after treatment. Interactions between herbicides were calculated by methods described by Colby (1967) (Hydrick and Shaw 1994; Koger et al. 2005; Koger et al. 2007). This method compares observed percent reduction values of herbicide combinations to expected percent reduction values calculated from percent reduction of the herbicides applied alone.

Expected percent reduction values are calculated as followed:

$$E= X\text{-}Y\ (XY/100) \qquad (3\text{-}1)$$

where E is the expected value, X is equal to the percent inhibition of growth by herbicide A at p kg ha^{-1}, and Y is equal to the percent inhibition of growth by herbicide B at p kg ha^{-1}. Expected and observed values were compared by Fisher's protected LSD at the 0.05 level of significance. If the observed response was greater than the expected response, then the herbicide combination was considered synergistic. If the observed response was less than the expected response, then the herbicide combination was considered antagonistic. Tank-mix combinations that produced no difference in the observed and expected response were considered additive.

^{14}C-Dicamba Absorption and Translocation Study

Barnyardgrass plants for these studies were established and grown as previously described. Plants were selected for treatment when the second true leaf was fully developed (15-20 cm in height). Adhesive-backed paper, 2.5 cm wide, was place over the second true leaf approximately 2.5 cm from the collar region. Plants were sprayed with nonradiolabeled rates of dicamba and dicamba/glyphosate herbicide combinations at the following rates: (1) 0.28 kg ha^{-1} dicamba plus 0.5% (v/v) 391A; (2) 0.28 kg ha^{-1} dicamba plus 0.28 kg ha^{-1} glyphosate; (3) 0.28 kg ha^{-1} dicamba plus 0.84 kg ha^{-1} glyphosate; (4) 0.56 kg ha^{-1} dicamba plus 0.5% (v/v) 391A; (5) 0.56 kg ha^{-1} dicamba plus 0.28 kg ha^{-1} glyphosate; and (6) 0.56 kg ha^{-1} dicamba plus 0.84 kg ha^{-1} glyphosate. All plants were sprayed with use of a compressed air spray chamber equipped with an XR110015E flat fan nozzle at an application volume of 169 L ha^{-1}. Immediately following spraying, ^{14}C-dicamba was applied to the area covered during spraying. The ^{14}C-dicamba solution was prepared by dissolving ^{14}C-dicamba (^{14}C[U-benzene]- labeled ring with 2.87 MBq/mg specific activity, 97.45% radiochemical purity) in an aqueous solution of dicamba and 391A or dicamba plus glyphosate combination. A 10 µl volume of the final ^{14}C-dicamba solution was placed on the adaxial surface of the second fully expanded true leaf with a 10-µl pipette. To ensure the applied ^{14}C-dicamba solution remained on the treated leaf, chenille strips were used to stabilize the leaf in a horizontal position and lanolin barriers were placed transverse to the edge of each treated zone (Dodds et al. 2007).

Plants were harvested 4, 12, 24, and 72 h after treatment with ^{14}C-dicamba. The treated portion of the leaf containing the ^{14}C-dicamba was excised and ^{14}C-dicamba remaining on the leaf was washed in 10 ml deionized water for 15 s. The treated sample was then washed in 10 ml of chloroform for 15 s to remove ^{14}C-dicamba from the epicuticular wax. After washing, the treated sample was placed in an empty glass scintillation vial and lyophilized. Once the treated section was removed, plants were then divided into the following fractions to determine translocation patterns: leaf tissue from treated area to leaf tip, leaf tissue from treated area to collar of treated leaf, plant material from collar of treated leaf to plant tip, plant material from collar of treated leaf to soil line, and roots. Plant fractions, other than the treated area, were placed in paper coin envelopes and lyophilized. A 1-ml aliquot was withdrawn from each rinsate and mixed with 10 ml of liquid scintillation cocktail for quantification by liquid scintillation spectrometry. Plant samples were combusted utilizing a biological oxidizer and evolved CO_2 was captured in 10 ml of liquid scintillation cocktail. Radioactivity in leaf washes and oxidations was determined by liquid scintillation spectrometry with internal quench and automatic quench correction. The sum of the ^{14}C recovered from the leaf washes and oxidations was considered the amount of ^{14}C recovered. Recovered ^{14}C was expressed as percent of total applied ^{14}C. Radioactivity located in plant fractions was considered absorbed. Radioactivity recovered from plant fractions other than the treated leaf was considered translocated.

Treatments were applied in a two factor factorial arrangement in a randomized complete block design: factor A was dicamba plus glyphosate rate combination, factor B was time after application. Each treatment consisted of four replicates and the experiment was repeated. Data were combined over experiment, analyzing experiment as a random effect, because of no interaction. Data were also pooled across herbicide rate combination and time where no interaction was observed. Data were subjected to an analysis of variance with means separated by Fisher's protected least significance difference (LSD) at the 0.05 level of probability (Dodds et al. 2007).

Results and Discussion

Interaction Study

Barnyardgrass fresh weight reduction ranged from 24 to 93% with glyphosate and 11 to 16% with dicamba (Table 3.1). Antagonism occurred when 0.14 and 0.28 kg ha^{-1} dicamba were combined with either 0.84 or 1.12 kg ha^{-1} glyphosate. The combination of 0.42 kg ha^{-1} dicamba and 1.12 kg ha^{-1} glyphosate also provided an antagonistic effect. The aforementioned tank-mix combinations all reduced control of barnyardgrass compared to glyphosate alone. However, combinations of 0.28 and 0.42 kg ha^{-1} dicamba with either 0.28 or 0.56 kg ha^{-1} glyphosate, as well as, 0.56 kg ha^{-1} dicamba tank-mixed with either 0.28, 0.56, or 0.84 kg ha^{-1} glyphosate all improved control of barnyardgrass, resulting in a synergistic effect. Flint and Barrett (1989b) reported that antagonism from glyphosate tank-mix combinations with either 2,4-D or dicamba could be overcome by increasing the rate of glyphosate applied. Similarly, O'Sullivan and O'Donovan (1980) reported that antagonism resulting from tank-mix combinations of glyphosate and dicamba could be overcome by sequential applications of the herbicide combination.

^{14}C-Dicamba Absorption and Translocation Study

Recovery levels of ^{14}C-dicamba in barnyardgrass ranged from 92 to 96% (Table 3.2), with no differences between herbicide treatments or time after application. The majority of the recovered ^{14}C-dicamba was located in the deionized water wash. The highest level of nonabsorbed ^{14}C-dicamba located in the wash was obtained with 0.56 kg ha^{-1} dicamba applied alone, at 72% of applied ^{14}C. Radioactivity in the water wash was greatest 4 h after application and decreased exponentially out to 72 h (Table 3.3).   There were no observed differences in recovered ^{14}C from the chloroform washes for herbicide treatment or time after application, indicating herbicide combination was not bound by epicuticular wax on the leaf surface. The lowest level of absorption resulted from the application of 0.56 kg ha^{-1} dicamba, which offset the high level of nonabsorbed ^{14}C observed with 0.56 kg ha^{-1} dicamba (Table 3.2). Absorption of

^{14}C-dicamba increased from 9 to 74% with time after application, out to 72 h, corresponding to decreased ^{14}C located in the water wash as time increased (Table 3.3). The relative translocation of ^{14}C-dicamba from the percent absorbed exhibited no difference with regard to herbicide treatment (Table 3.2). However, Flint and Barrett (1989b) reported a decrease in relative translocation of ^{14}C-glyphosate in johnsongrass when tank-mixed with dicamba compared with glyphosate alone. Highest levels of relative translocation with regard to time after application were reported 12 to 72 h after treatment. These results are similar to those of Chang and Vanden Born (1971) and Magalhaes et al. (1968) that reported increased translocation of dicamba with respect to time after application.

Tank-mix combinations of glyphosate and dicamba resulted in altered translocation patterns of absorbed ^{14}C-dicamba across all barnyardgrass partitions (Table 3.4). Dicamba applied alone at 0.56 kg ha^{-1} provided the lowest level of recovered radioactivity from the treated area of the leaf. There were no differences among other herbicide treatments with regards to radioactivity located in the treated area: however, data indicated a gradual increase, from 6 to 49%, in amount of recovered ^{14}C in treated area as time after application increased from 4 to 72 h (Table 3.3). The same trend was observed with amount of recovered ^{14}C-dicamba located in all nontreated, above-ground plant fractions with timings of 4, 12, 24, and 72 h, which contained 2, 8, 13, and 22 % of applied ^{14}C, respectively. Chang and Vanden Born (1971) reported increasing concentrations of ^{14}C-dicamba recovered from non-treated plant parts of barley and wheat out to 20 days. Leaf tissue from treated area to leaf tip exhibited the lowest levels of radioactivity when 0.56 kg ha^{-1}dicamba was applied alone compared to tank-mix combinations with glyphosate. (Table 3.4). Leaf tissue from the treated area of the leaf to the collar produced the lowest levels of ^{14}C recovery when 0.28 kg ha^{-1} dicamba was tank-mixed with 0.28 kg ha^{-1} of glyphosate or when 0.56 kg ha^{-1}dicamba was applied alone.

The addition of glyphosate to herbicide treatments caused a decrease in amount of radioactivity recovered from collar of treated leaf to plant tip when compared to dicamba alone 72 h after initial application. The accumulation of dicamba in young leaves corresponds with Chang and Vanden Born (1971) data indicating a propensity for dicamba to accrue in the tips of wheat plants. Higher levels of ^{14}C-dicamba accumulation from the collar of the treated leaf to the soil line were also observed 72 h after treatment when dicamba was applied alone. Regardless of herbicide application, radioactivity recovered from roots was minimal. However, concentrations of applied ^{14}C-dicamba increased from 0.04 to 0.38%, in a step-wise manner, as time of application increased from 4h to 72 h (Table 3.3). Magalhaes et al. (1968) also reported very low amounts of detectable ^{14}C-dicamba located in the roots of purple nutsedge (*Cyperus rotundus* L.).

The results of this study indicate varying responses of barnyardgrass to tank-mix combinations of dicamba and glyphosate. Flint and Barrett (1989b) indicated that reduction in absorption and translocation in johnsongrass, from glyphosate and dicamba combinations, could be overcome by increasing the rate of glyphosate applied. The reduction of barnyardgrass control in this study, from tank-mix combinations of glyphosate and dicamba, did not appear to be a product of decreased absorption or relative translocation of dicamba. However, combinations of glyphosate and dicamba resulted in decreased amounts of ^{14}C-dicamba recovered over time in above-ground plant material located away from the treated leaf. Dicamba is transported in the plant both symplastically and apoplastically, resulting in accumulation at growing points (WSSA 2007). A reduction in dicamba partitioned in growing points, resulting from tank-mix combinations with glyphosate, could indicate a physiological effect limiting the herbicide translocation. Auxin-like herbicides can cause swelling of the stem and petiole resulting in physical constriction in both the xylem and the phloem (Peterson et al. 1974). Blockage of plant transport systems could prevent toxic quantities of certain herbicides from reaching roots and growing points (Devine et al. 1993). The ability of a species to be resistant or susceptible to dicamba depends on selective uptake, translocation, and metabolism (Chang and Vanden Born 1971). Geiger and Bestman (1990) reported that glyphosate has the ability to interfere with its own translocation by interfering with carbon transport and metabolism effectively preventing the establishment of phytotoxic levels in sink organs. Observed reduction in translocation appears to result from interference with synthesis of aromatic amino acids or enzymes needed in sink leaf metabolism (Geiger and Bestman 1990; Gougler and Geiger 1981).

Decreased control exhibited from glyphosate/dicamba combinations could be overcome by increasing the dicamba rate to 0.56 kg ha^{-1}. However, this research has shown that there are many variables that can impact the effectiveness of glyphosate and dicamba tank-mix combinations. Further research should be conducted to understand the full effect of glyphosate in tank-mix combination with dicamba.

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Table 3.1. Percent reduction in fresh weight of barnyardgrass 21 days after postemergence treatments with tank-mix combinations of dicamba and glyphosate.

Glyphosate rate	Dicamba rate (kg ae/ha) [a]				
	0.0	0.14	0.28	0.42	0.56
(kg ae/ha)	%				
0.0		11	16	16	14
0.28	24	27 (32) [b]	54 (36)+ [c]	80 (36)+	94 (34)+
0.56	25	32 (32)	53 (37)+	80 (37)+	95 (35)+
0.84	75	39 (78)-	47 (79)-	81 (79)	95 (78)
1.12	93	48 (94)-	52 (95)-	80 (94)-	96 (94)
LSD [d]	7				

[a] Dicamba treatment applied alone contained 0.05% 391A.

[b] Values in parentheses are the calculated (expected) level of percent fresh weight reduction for the herbicide combinations.

[c] A negative sign denotes an antagonistic response; a positive sign denotes a synergistic response.

[d] LSD: Least significant difference separated by Fishers protected LSD at the 0.05 level of significance

Table 3.2. Effect of dicamba plus glyphosate rate combinations on absorption of ^{14}C-dicamba in barnyardgrass.

Herbicide treatment	Rate (kg ae/ha)	^{14}C in leaf wash: Water	^{14}C in leaf wash: Chloroform	Absorption	Recovery	Relative Translocation
		----% of applied----				-----% of absorbed-----
dicamba	0.28	61 [a]	0.25	34	95	36
dicamba + glyphosate	0.28 + 0.28	58	0.45	34	92	34
dicamba + glyphosate	0.28 + 0.84	57	0.26	35	92	36
dicamba	0.56	72	0.22	24	96	38
dicamba + glyphosate	0.56 + 0.28	61	0.29	35	96	39
dicamba + glyphosate	0.56 + 0.84	59	0.30	34	93	39
LSD (0.05) [c]		7	NS [b]	6	NS	NS

[a] ^{14}C-dicamba distribution is based on percentage of ^{14}C-dicamba absorbed over 4, 12, 24, and 7h after treatment.

[b] NS: No significant difference among treatments.

[c] LSD: Least significant difference separated by Fishers protected LSD at the 0.05 level of significance

Table 3.3. Effect of time after application on partitioning of ^{14}C-dicamba in barnyardgrass.

	^{14}C in leaf wash		^{14}C in plant fractions				
Time after application	Water	Chloroform	Treated leaf	Remaining leaves	Roots	Absorption	Relative translocation
h	-------------------------	--------	-------% of applied------	--------	--------	--------	-----% of Absorbed-----
4	86 [a]	0.34	6	2	0.04	9	29
12	71	0.27	12	8	0.09	22	38
24	59	0.31	20	13	0.17	36	41
72	14	0.25	49	22	0.38	74	37
LSD (0.05) [c]	6	NS [b]	3	3	0.06	6	5

[a] ^{14}C-dicamba distribution is based on percentage of ^{14}C-dicamba absorbed over all herbicide treatments.

[b] NS: No significant difference among treatments.

[c] LSD: Least significant difference separated by Fishers protected LSD at the 0.05 level of significance

Table 3.4. Effect of dicamba plus glyphosate rate combinations on partitioning of ^{14}C-dicamba in barnyardgrass.

Herbicide treatment	Rate (kg ae/ha)	^{14}C in plant fractions: Treated Area	Leaf tissue from treated area to leaf tip	Leaf tissue from treated area to collar	[d]Plant material from collar of treated leaf to plant tip [d]: 4 h	12 h	24 h	72 h	[d]Plant material from collar of treated leaf to soil line: 4 h	12 h	24 h	72 h	Roots
		% of applied											
dicamba	0.28	23 [a]	5	3	0.06	0.16	0.94	2.73	0.61	1.40	2.39	4.16	0.20
dicamba + glyphosate	0.28 + 0.28	23	7	2	0.07	0.31	0.87	0.71	0.49	0.98	1.99	2.18	0.14
dicamba + glyphosate	0.28 + 0.84	24	6	3	0.04	0.36	0.70	0.66	0.52	2.18	1.64	1.84	0.14
dicamba	0.56	15	4	2	0.03	0.27	0.42	1.96	0.29	0.78	1.49	4.06	0.17
dicamba + glyphosate	0.56 + 0.28	22	7	3	0.04	0.37	1.07	0.91	0.31	2.50	3.20	2.06	0.19
dicamba + glyphosate	0.56 + 0.84	24	7	3	0.05	0.20	0.56	0.66	0.46	2.17	2.31	2.08	0.17
LSD (0.05) [c]		5	2	1	0.47				1.50				NS [b]

[a] ^{14}C-dicamba distribution is based on percentage of ^{14}C-dicamba absorbed over 4, 12, 24, and 7h after treatment.

[b] NS: No significant difference among treatments.

[c] LSD: Least significant difference separated by Fishers protected LSD at the 0.05 level of significance.

[d] Results separated by time after application (indicated as 4,12,24 and 72 h) because of significant ($P \leq 0.05$) time X herbicide interaction.

CHAPTER IV

EFFECTS OF GLYPHOSATE AND DICAMBA TANK-MIX COMBINATIONS ON EFFICACY, ABSORPTION, AND TRANSLOCATION IN SICKLEPOD (*Senna obtusifolia*)

Abstract

Greenhouse studies were conducted to determine the effects of dicamba/glyphosate tank-mix combinations on sicklepod, and identify the basis for any observed response. Sicklepod was treated at the 4±1 leaf stage with various rates of dicamba, glyphosate, and tank-mix combinations of the two herbicides. Antagonistic responses were observed when 0.28 and .56 kg ae ha^{-1} glyphosate were combined with 0.14, 0.28, or 0.42 kg ae ha^{-1} dicamba, as well as the tank-mix of 0.56 kg ae ha^{-1} glyphosate and 0.56 kg ae ha^{-1} dicamba. The combinations of 0.28 and 0.56 kg ha^{-1} glyphosate with either 0.14 or 0.28 kg ha^{-1} dicamba reduced control of sicklepod compared with both glyphosate and dicamba applied alone. Antagonism was no longer observed with tank-mix combinations when glyphosate rates were increased to 0.84 or 1.12 kg ha^{-1}. Rates were selected from the interaction study to observe the effects of dicamba tank-mix combinations on absorption and translocations of ^{14}C-dicamba. Dicamba, glyphosate, and tank-mix combinations were sprayed on sicklepod plants before treatment with ^{14}C-dicamba. Plants were harvested 4, 12, 24, and

72 h after treatment. The addition of glyphosate to dicamba resulted in reduced translocation of recovered ^{14}C-dicamba; the combination resulted in a concomitant increase in recovery from the treated leaf. ^{14}C-dicamba concentrations increased incrementally over time within all observed plant fractions, regardless of herbicide combination or rate. These data indicate that increasing the rate of glyphosate in combination with dicamba effectively overcame antagonism on sicklepod, despite the observed reduction in dicamba absorption and translocation.

Nomenclature: dicamba, glyphosate, sicklepod, [*Senna obtusifolia* (L.) H.S. Irwin & Barneby]

Introduction

Dicamba is a synthetic auxin herbicide used for control of broadleaf weeds in a variety of crops (Bradley et al. 2003; Everitt and Keeling 2007; Rinella et al. 2001). Dicamba mimics the natural plant hormone indole-3-acetic acid, causing an epinastic response in target weed species, eventually leading to chlorosis and necrosis (WSSA 2007). The development of glyphosate-resistant (GR) weeds has resulted in the promotion of increased diversity in weed control programs, including alternative modes of action, to help decrease the spread and development of resistant populations (Koger et al. 2005; Mueller et al. 2005; Powles 2003). Dicamba has been used to control GR populations of horseweed [*Conyza canadensis* (L.) Cronq.] (Owen et al. 2009) and Palmer amaranth (*Amaranthus palmeri* S. Wats.) (Norsworthy et al. 2008) and is labeled for control of giant ragweed (*Ambrosia trifida* L.), common ragweed (*Ambrosia artemisiifolia* L.), kochia [*Kochia scoparia* (L.) Schrad.], and common waterhemp (*Amaranthus rudis* Sauer) (BASF 2010).

Recently, biotechnological advancements have been made to include dicamba tolerance in cotton (*Gossypium hirsutum* L.) and soybean [*Glycine max* (L.) Merr.] (Behrens et al. 2007). Dicamba crop tolerance allows applications of dicamba alone or in combination with other herbicides, including glyphosate, to be made without risk of crop injury (Behrens et al. 2007). While this technology sounds very promising, tank-mix combinations involving dicamba, glyphosate, and several other herbicides have produced varied results in a number of weed species. Koger et al. (2005) showed increased control of pitted morningglory (*Ipomoea lacunosa* L.) and hemp sesbania [*Sesbania herbacea* (P.Mill) McVaugh] when trifloxysulfuron and glyphosate were applied together compared with glyphosate alone. However, Koger et al. (2007) found that combinations of MSMA with glyphosate caused antagonistic effects on barnyardgrass [*Echinochloa crus-galli* (L.) Beauv.] , browntop millet *[Urochloa ramosa* (L.) Nguyen], hemp sesbania, Palmer amaranth, and redroot pigweed (*Amaranthus retroflexus* L.) Flint and Barrett (1989a) reported synergistic effects on field bindweed (*Convolvulus arvensis* L.) when glyphosate and dicamba were combined. Antagonistic effects were observed on graminaceae species when dicamba was applied with imazethapyr (Hart and Wax 1996) and glyphosate (Flint and Barrett 1989b). Glyphosate combined with dicamba may be effective for controlling many weed species. However, the potential for antagonism still must be assessed. One species that has seen varied responses to tank-mix applications of various herbicides is sicklepod (Waldrop and Banks 1983).

Sicklepod is an annual broadleaf distributed throughout most of the eastern United States and part of southern California (SWSS 2009). Webster (2001) reported sicklepod as one of the 10 most troublesome weeds in the southeastern United States. The timing, rate, and mode of action of herbicide chosen to control sicklepod can influence management strategies from one year to the next (Wixson and Shaw 1991; Ratnayake and Shaw 1992). Corbett et al. (2004) reported complete control of sicklepod with glyphosate when applied from 2 to 5 cm. Koger et al. (2005) showed control of sicklepod when glyphosate was applied at 0.84 kg ha^{-1} during both early- and late-postemergence timings.

Dicamba is labeled for sicklepod control (BASF 2010), but there is no data on the effects of dicamba plus glyphosate tank-mix combinations on sicklepod efficacy. The objective of this study was to determine the potential of dicamba and glyphosate tank-mix combinations on sicklepod efficacy and identify the basis for any observed response with dicamba.

Materials and Methods

Interaction Study

Greenhouse experiments were conducted at the R.R. Foil Plant Science Research Center, Mississippi State University, in 2009. Sicklepod seed was planted in a 9 cm^2 pots containing Metro-Mix 300 (horticulture grade vermiculite, bark, Canadian sphagnum peat moss, horticulture grade perlite, processed bark ash, starter nutrient charge, dolomitic limestone, and wetting agent). Temperature in the greenhouse was maintained at 35/30 C day/night and photoperiod was approximately 16-h; supplemental lighting was provided by sodium vapor lamps. Plants were thinned to one plant per pot within one week of germination and surface irrigated daily to provide adequate moisture.

The experiment was conducted twice in a randomized complete block design with a two-factor factorial arrangement of treatments, consisting of four replications. The first factor consisted of 0.14, 0.28, 0.42, and 0.56 kg ha^{-1} of the diglycolamine salt of dicamba. The second factor consisted of 0.28, 0.56, 0.84, and 1.12 kg ha^{-1} of the isopropyl amine salt of glyphosate. Herbicide rates were selected based on label rates and prior experiments involving glyphosate plus dicamba tank-mix combinations (Flint and Barrett 1989a; Flint and Barrett 1989b). Plants were selected for treatment upon full development of the second node. All herbicide rates were applied individually and in combinations in a compressed air spray chamber equipped with an XR110015E flat fan nozzle at 169 L ha^{-1}. Dicamba treatments applied individually included 391A, a proprietary surfactant, at 0.5% (v/v) to equalize surfactant effects. Plants were harvested and fresh weight taken 21 days after treatment. Expected values for percent control of herbicide combinations were calculated using methods described by Colby (1967) (Hydrick and Shaw 1994; Koger et al. 2005; Koger et al. 2007). This method compares observed percent reduction values of herbicide combinations to expected percent reduction values calculated from percent reduction of the herbicides applied alone.

Expected percent reduction values are calculated as followed:

$$E= X-Y\ (XY/100) \tag{4-1}$$

where E is the expected value, X is equal to the percent inhibition of growth by herbicide A at p kg ha^{-1}, and Y is equal to the percent inhibition of growth by herbicide B at p kg ha^{-1}. Interactions between the observed and expected values were compared using Fisher's protected LSD at the 0.05 level of significance. If the observed response was higher or lower than the expected response, the combination was considered synergistic or antagonistic, respectively.

^{14}C-Dicamba Absorption and Translocation Study

Sicklepod plants for these studies were established and grown as previously described. Plants were selected when the second node was fully developed (5 to 10 cm). Adhesive backed paper was placed over the first leaf on the left hand side of the second node. Plants were presprayed with nonradiolabeled rates of dicamba and dicamba/glyphosate herbicide combinations at the following rates: (1) 0.28 kg ha^{-1} dicamba plus 0.5% (v/v) 391A; (2) 0.28 kg ha^{-1} dicamba plus 0.28 kg ha^{-1} glyphosate; (3) 0.28 kg ha^{-1} dicamba plus 0.84 kg ha^{-1} glyphosate; (4) 0.56 kg ha^{-1} dicamba plus 0.5% (v/v)

391A; (5) 0.56 kg ha^{-1} dicamba plus 0.28 kg ha^{-1} glyphosate; and (6) 0.56 kg ha^{-1} dicamba plus 0.84 kg ha^{-1} glyphosate.

All plants were presprayed with use of a compressed air spray chamber with an XR110015E flat fan nozzle at an application volume of 169 L ha^{-1}. Immediately following prespraying, ^{14}C-dicamba was applied to the area covered during prespraying. The ^{14}C-dicamba solution was prepared by dissolving ^{14}C-dicamba (^{14}C[U-benzene]- labeled ring with 2.87 MBq/mg specific activity, 97.45% radiochemical purity) in an aqueous solution of dicamba and 391A or dicamba/glyphosate combination. A 10-μl volume of the final ^{14}C-dicamba solution was placed on the adaxial surface of the first leaf on the left hand side of the second node with a 10-μl pipette. To ensure the applied ^{14}C-dicamba solution remained on the treated leaf, chenille strips were used to stabilize the leaf in a horizontal position.

Plants were harvested 4, 12, 24, and 72 h after treatment with ^{14}C-dicamba. The treated leaf was excised, and ^{14}C-dicamba remaining on the leaf surface was removed by washing in 10 ml of deionized water for 15 s. Next, the treated portion of the leaf was washed in 10-ml of chloroform for 15 s to remove ^{14}C-dicamba from the epicuticular wax. After washing with chloroform, the treated leaf was placed in a glass scintillation vial and lyophilized. Plants were then partitioned into the following sections: plant material above treated node, second internode and leaves (minus treated leaf), first internode and leaves, hypocotyl and cotyledon, and roots. Plant fractions, other than the treated area, were placed in paper coin envelopes and lyophilized. A 1-ml aliquot was withdrawn from each rinsate and mixed with 10 ml of liquid scintillation cocktail for quantification by liquid scintillation spectrometry. Plant samples were combusted utilizing a biological oxidizer and evolved CO_2 was captured in 10 ml of liquid scintillation cocktail. Radioactivity in leaf washes and oxidations was determined by liquid scintillation spectrometry with internal quench and automatic quench correction. The sum of the ^{14}C recovered from the leaf washes and oxidations was considered the amount of ^{14}C recovered. Radioactivity located in plant fractions was considered absorbed. Radioactivity recovered from plant fractions other than the treated leaf was considered translocated.

Treatments were applied in a two-factor factorial arrangement in a randomized complete block design: factor A was dicamba plus glyphosate rate combination, factor B was time after application. Each treatment consisted of four replicates and the experiment was replicated. Data were combined over experiment, analyzing experiment as a random effect, because of no interaction. Data were also pooled across herbicide rate combination and time where no interaction was observed. Data were subjected to an analysis of variance with means separated by Fisher's protected least significance difference (LSD) at the 0.05 level of probability.

Results and Discussion

Interaction Study

Sicklepod fresh weight reduction ranged from 53 to 100% with glyphosate and 65 to 66% with dicamba (Table 4.1). Antagonism occurred when 0.28 and 0.56 kg ha^{-1} glyphosate were combined with 0.14, 0.28, or 0.42 kg ha^{-1} dicamba. The combination of 0.56 kg ha^{-1} glyphosate and 0.56 kg ha^{-1} dicamba also provided an antagonistic effect. Tank-mix combinations of 0.28 and 0.56 kg ha^{-1} glyphosate with either 0.14 or 0.28 kg ha^{-1} dicamba reduced control of sicklepod compared to both glyphosate and dicamba applied alone. Reduced control of sicklepod was also observed with 0.42 and 0.56 kg ha^{-1} dicamba combined with 0.56 kg ha^{-1} glyphosate compared with glyphosate alone, and 0.42 kg ha^{-1} dicamba with 0.28 kg ha^{-1} glyphosate when compared with dicamba alone. Increasing the rate of glyphosate to 0.84 or 1.12 kg ha^{-1} eliminates any antagonistic response and results in an additive effect of the herbicide combination. Similarly, Hydrick and Shaw (1994) reported that increased rates of nonselective herbicides in combination with residual herbicides eliminated antagonistic responses observed with reduced rates.

^{14}C-Dicamba Absorption and Translocation Study

Radiolabeled dicamba recovery levels ranged from 81 to 99% (Table 4.2). Low recovery rates for some levels could have resulted from contamination issues associated with the biological oxidizer utilized in the test. Missing data points were used to account for the contaminated samples. Data were then combined over experiments and values expressed as percent of recovered ^{14}C . The lowest levels of absorption resulted from the application of 0.56 kg ha^{-1} dicamba 4 and 12 h after treatment. However, absorption levels of ^{14}C-dicamba increased when glyphosate was added at 0.28 or .84 kg ha^{-1} 4 and 12 h after treatment. Incremental increases in absorption levels ranging from 37 to 97% of recovered ^{14}C-dicamba were also observed as time of application increased from 4 to 72 h. Relative translocation of radioactivity was highest when dicamba was applied alone at 0.28 or 0.56 kg ha^{-1}. The addition of either 0.28 or 0.84 kg ha^{-1} glyphosate to either dicamba rates decreased relative translocation of ^{14}C-dicamba. Flint and Barrett (1989a) also reported increased absorption of ^{14}C-dicamba when glyphosate was added, but decreases in relative translocation of the same treatments.

An increase in accumulation of ^{14}C-dicamba in the treated leaf occurred when both 0.28 and 0.84 kg ha^{-1} glyphosate were added to either dicamba rate (Table 4.2). Conversely, these same herbicide combinations reduced the amount ^{14}C-dicamba located in nontreated leaves on the plant compared with both 0.28 and 0.56 kg ha^{-1} dicamba applied alone. Concentrations of percent of recovered radioactivity decreased in treated leaves and increased in nontreated leaves as time after application increased, regardless of herbicide treatment (Table 4.3). The decrease in radioactivity from 80 to 40 % in the treated leaf, ranging from 4 to 72 h after treatment, is similar to the one day or more translocation from the treated leaf observed by Chang and Vanden Born (1971) in Tartary buckwheat

[*Fagopyrum tataricum* (L.) Gaertn.] and wild mustard (*Sinapis arvensis* L.). Radioactivity increased in the above-ground fraction when glyphosate was tank-mixed with either rate of dicamba (Table 4.2). The absence of glyphosate with 0.56 kg ha^{-1} dicamba resulted in greater retention of ^{14}C-dicamba in the roots. Accumulation of ^{14}C-dicamba increased in above-ground fractions and decreased in roots with increases in time of application (Table 4.3).

Relative changes in absorption and translocation of ^{14}C-dicamba may explain the reason for observed antagonism with dicamba and low rates of glyphosate in sicklepod. Flint and Barrett (1989a) reported the possibility that glyphosate movement may have been hindered by the addition of dicamba. This hypothesis is based on the ability of glyphosate to limit its own movement by disrupting carbon metabolism (Gougler and Geiger 1984). Gieger and Bestman (1990) reported that the inclusion of compounds whose effectiveness is based on phloem mobility in herbicide mixtures should generally be avoided. Flint and Barrett (1989a) also reported increased uptake of 0.28 kg ha^{-1} glyphosate in the treated leaf when combined with dicamba, but as glyphosate rate increased, dicamba no longer affected the percentage recovered. The interaction study exhibited an antagonistic response when low rates of glyphosate were applied with dicamba. The relative translocation of dicamba applied alone was higher than tank-mix combinations of glyphosate and dicamba. This supports findings of the Geiger and Bestman (1990), who reported the effects of phloem-mobile herbicides limiting the import of herbicides into sink tissues in the plant. However, the hypothesis may stand that increasing levels of nonselective herbicides can be enough to overcome reductions in translocation and provide phytotoxic levels to target species.

Altered translocation of glyphosate has been reported as an identifier for glyphosate resistance in horseweed (Koger and Reddy 2005). The altered translocation exhibited in these data combined with the aforementioned translocation data on horseweed raise further questions about the ability of tank-mix combinations of glyphosate plus dicamba to control glyphosate resistant populations. These results suggest the possibility that dicamba could see altered translocation patterns in resistant species resulting in reduced control.

On the basis of these data, the combination glyphosate and dicamba does cause antagonism to occur in sicklepod with reduced rates of glyphosate compared with glyphosate and dicamba alone. However, increasing glyphosate rate to at least 0.84 kg ha^{-1} reestablishes control of sicklepod similar to control levels observed with glyphosate alone and higher than control levels observed with dicamba alone. The reduction of absorption and translocation of dicamba observed with the tank-mix seems to indicate a physiological response in the plant. Behavior of these tank-mix combinations and the physiological basis for the observed tank-mix interaction between glyphosate and dicamba should warrant further research.

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Table 4.1. Percent reduction in fresh weight of sicklepod 21 days after postemergence treatments with tank-mix combinations of dicamba and glyphosate.

Glyphosate	Dicamba rate (kg ae/ha) [a]				
rate	0.0	0.14	0.28	0.42	0.56
(kg ae/ ha)	%				
0.0		65	66	66	65
0.28	53	49 (83)- [b,c]	44 (84)-	53 (84)-	76 (83)
0.56	99	49 (99)-	55 (100)-	63 (100)-	82 (100)-
0.84	100	100 (100)	100 (100)	100 (100)	100 (100)
1.12	100	100 (100)	100 (100)	100 (100)	100 (100)
LSD [d]	10				

[a] Dicamba treatment applied alone contained 0.05% 391A.

[b] Values in parentheses are the calculated (expected) level of percent fresh weight reduction for the herbicide combinations.

[c] A negative sign denotes an antagonistic response; a positive sign denotes a synergistic response.

[d] LSD: Least significant difference separated by Fishers protected LSD at the 0.05 level of significance

Table 4.2. Effect of dicamba plus glyphosate rate combinations on partitioning of ^{14}C-dicamba in sicklepod.

		^{14}C in plant fractions									
	Rate	Percent	Absorption [c]				Relative	Treated	Non Treated	Above ground	
Herbicide treatment	(kg ae/ha)	recovery	4 h	12 h	24 h	72 h	translocation	leaf	Leaves	fraction	Roots
		% of Recovered									
dicamba	0.28	81 [a]	75	75	78	97	50	50	48	98	2
dicamba + glyphosate	0.28 + 0.28	81	89	91	93	97	36	64	35	99	2
dicamba + glyphosate	0.28 + 0.84	85	77	89	91	97	35	65	34	99	1
dicamba	0.56	85	37	53	82	84	45	55	42	97	3
dicamba + glyphosate	0.56 + 0.28	99	76	88	93	96	22	78	21	99	1
dicamba + glyphosate	0.56 + 0.84	89	73	90	88	96	22	77	21	99	2
LSD (0.05) [b]		8	13				8	8	8	1	1

[a] ^{14}C-dicamba distribution is based on percentage of ^{14}C-dicamba recovered over 4, 12, 24, and 72h after treatment.

[b] LSD: Least significant difference separated by Fishers protected LSD at the 0.05 level of significance

[c] Results separated by time after application (indicated as 4,12,24 and 24 h) because of significant ($P \leq 0.05$) time X herbicide interaction.

Table 4.3. Effect of time after application on partitioning of ^{14}C-dicamba in sicklepod.

		^{14}C in plant fractions			
Time after application	Relative Translocation	Treated Leaf	Nontreated leaves	Above ground fraction	Roots
h	-----------------------	-----------------------	% of Recovered	-----------------------	-----------------------
4	20 [a]	80	17	97	2.74
12	23	77	20	97	2.62
24	36	63	35	98	1.73
72	60	40	60	99	0.51
LSD (0.05) [b]	7	7	7	1	1

[a] ^{14}C-dicamba distribution is based on percentage of ^{14}C-dicamba recovered over all herbicide treatments.

[b] LSD: Least significant difference separated by Fishers protected LSD at the 0.05 level of significance